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On the Nature and Physiological Action of the *Crotalus*-poison as compared with that of *Naja tripudians* and other Indian Venomous Snakes ; also Investigations into the Nature of the Influence of *Naja*- and *Crotalus*-poison on Ciliary and Amœboid Action and on *Vallisneria*, and on the Influence of Inspiration of pure Oxygen on Poisoned Animals. By T. LAUDER BRUNTON, M.D., F.R.S., Sc.D., M.R.C.P., and J. FAYRER, C.S.I., M.D., F.R.C.P. Lond., F.R.S.E., President of the Medical Board at the India Office.

In our former papers we described the general phenomena accompanying the physiological action of cobra- and *Daboia*-poisons on warm-blooded animals, reptiles, fishes, and invertebrata. We propose in this

paper to compare with these the action of the *Crotalus*-virus in its general effects on life, on the functions, organs, and tissues, and especially as it affects the blood and vessels as regards a marked influence in causing hæmorrhages and extravasations of blood generally and locally; and, further, to examine the action of snake-poison generally on ciliary and amoeboid movements—or that which represents its action on contractility, apart from that which is caused through the medium of the nerve-centres and nerve-distribution.

It appears that there is little difference between the physiological effects of the crotaline or viperine and the colubrine virus. The mode in which death is brought about is essentially the same in all; though there are evidences, even when allowing for individual peculiarities, that the action is marked by some points of difference sufficiently characteristic to require notice in detail.

We have already expressed our belief that death is caused by the cobra-, *Daboia*-, and *Hydrophis*-poison, 1st, through its action on the cerebro-spinal nerve-centres, especially on the medulla, inducing paralysis of respiration; or 2nd, in some cases (where the poison has entered the circulation in large quantities and has been conveyed more directly to the heart) by arrest, tetanically in systole, of cardiac action, probably owing to some action on the cardiac ganglia; 3rd, by a combination of the two previous causes; 4th, by a septic condition of a secondary nature, and which, being more essentially pathological in its bearings, the details were not considered suitable for discussion here.

There is reason to believe that death is caused in the same way by the *Crotalus*-poison also; and it appears, from the experiments recently performed in Calcutta, by Dr. Ewart and the members of the Committee appointed by Government, upon *Pseudechis porphyriacus*, or the black snake, and *Hoplocephalus curtus*, or the tiger-snake of Australia, that their virus causes death in the same manner. These reptiles had been sent from Melbourne to Calcutta for the purpose of investigation and comparison. (*Vide* Committee's Report, p. 58 *et seq.*, Appendix.)

But though the actual cause of death is essentially the same, the phenomena which precede and accompany it differ in some degree according to the nature of the poison, the quantity and site of the inoculations, and the individual peculiarities of the creature inoculated, as may be seen in the experiments herewith recorded.

The condition of an animal poisoned by the rattlesnake-venom, then, essentially resembles that of one subjected to the influence of the colubrine or viperine poison of Indian snakes:—

Depression, hurried respiration, exhaustion, lethargy, unconsciousness, nausea, retching, and vomiting (*vide* experiment on cat, Exp. IX.).

Muscular twitchings, ataxy, paralysis, and convulsions (the latter probably chiefly, though not entirely, due to circulation of imperfectly oxygenated blood, the result of impeded respiration), and, finally, death.

Hæmorrhages or hæmorrhagic extravasations and effusions, both local and general, occur in all varieties of snake-poisoning.

But we observe (and in this our observations are in accord with those of Weir Mitchell) that there is a greater tendency to both local and general hæmorrhage and extravasation of blood and of the colouring-matter of the blood, especially as observed in the peritoneum, intestines, and mesentery, and also probably to a more direct action on the cord (*vide* Experiments I., III., V., VI., VII., IX., XI., XIV., XV.), than in poisoning by either cobra or viper (*vide* Experiments IV., VII., XIII., XVI., XVII., XX.).

The viscera and other tissues, after death, are found congested and ecchymosed, and in some cases to a great extent, seeming to show that either a preternatural fluidity of blood or some important change in the vessels, favouring its exudation, has occurred.

But with regard to the blood itself we have observed that it does form a coagulum after death, generally, if not invariably; as we have noted to be the case, though not to the same extent, in the blood of animals that have succumbed to the *Daboia*-virus\*.

With reference to the coagulation or non-coagulation of the blood in cases of snake-poisoning, we observe that the following conclusions have been arrived at by Mr. Richards and the Calcutta Committee (*vide* p. 45 of their Report).

“We now propose to deal with the physical changes produced by snake-poisoning on the blood. From observations which have been made by Mr. Richards and ourselves, we have arrived at the following conclusions.

“*The blood appears to remain fluid after death under the circumstances noted below:—*

“1st. When a large quantity of the cobra-poison has been directly injected into the circulation, as, for example, into an artery or a vein†.

“2nd. In cases where animals or men have been poisoned by the bite of vipers, such as the Russell's viper.

“3rd. In all cases of snake-bite, whether from the poisonous colubrine or viperine genera, in the human subject‡.

“*The blood undergoes either partial or complete coagulation under the following conditions:—*

“1st. When a small quantity only of the cobra-poison has been injected into a vein or an artery.

“2nd. In cases where the lower animals have been bitten by the cobra.

“Why the admixture of a large and quickly fatal injection of the cobra-virus into the circulation of animals should produce comparatively

\* In Dr. Fayrer's Indian experiments the blood of animals dead from *Daboia*-poison nearly always remained fluid after death.

† This is not always so.—J. Fayrer.

‡ Not always so.—J. Fayrer.



permanent fluidity of the blood or interfere with its ordinary coagulability soon after removal from the body or after death, and why the injection of a smaller and more slowly fatal quantity should interpose no obstacle to its speedy coagulation, are questions extremely difficult to account for or explain. We can only state the fact that, in the one case, coagulation occurs speedily, and in the other this coagulation is retarded or altogether prevented by some cause at present unknown."

The following experiments were made on the physiological action of the virus of the rattlesnake, with the view of comparison with that of the cobra and *Daboia*.

We are indebted to Dr. Weir Mitchell, of Philadelphia, for a supply of the virus. He was good enough to send about six grains of the dried poison of *Crotalus*—the species not named, but it is believed to be of *Crotalus durissus*.

The dried poison supplied is said to be about  $6\frac{1}{2}$  years old, and was dried in July or August at the natural temperature, and has since then been preserved in a phial. It was tried by Dr. Mitchell and found active three years ago.

It has the appearance of fractured fragments of dried gum-arabic, of rather a darker yellow colour, but otherwise resembling the dried cobra-virus sent from Bengal.

#### Experiment I.

June 9th, 1874.—0.15 gramme of the dried *Crotalus*-poison diluted with 1 cub. centim. of distilled water was hypodermically injected into the thigh of a full-grown guineapig at 11.30 A.M.

Restlessness and muscular twitchings of the body generally soon commenced; these passed away, but the animal became sluggish, in which condition it remained all night, and died at about 9 A.M. the next morning.

The injected limb became much swollen, infiltrated, and discoloured with sanguineo-serous effusion.

The intestines were not ecchymosed; there was much sanguinolent fluid and also blood effused into the abdominal areolar tissue.

No convulsions were observed; but as the animal was not seen during a short time previous to death, they cannot be said positively not to have occurred; nor is it known if the heart ceased to beat at the moment when apparent death took place.

#### Experiment II.

A few drops of watery solution of *Crotalus*-poison, of same strength, were injected under the skin of a guineapig's thigh at 12.16, noon.

12.17. Marked twitchings of head and hind legs, very similar to those produced in some of the cases of cobra-poisoning.

12.18. Hind leg (poisoned one) weak.

12.20. Twitchings much increased, now mainly in head and neck, not so much in hind legs.

12.28. Guinea-pig quiet, but with occasional twitchings; sluggish and disinclined to move.

1.30. Sluggish in moving; can still move about, though disinclined to do so. The punctured thigh is very blue.

The rest of the notes of this experiment were lost.

The animal died.

### Experiment III.

June 10th, 1874.— $\frac{1}{4}$  of a grain of *Crotalus*- and  $\frac{1}{4}$  of a grain of cobra-poison were carefully weighed and diluted, each with ten drops of distilled water. Two full-grown guinea-pigs of equal weight were then selected.

The solution of *Crotalus*-poison was injected into the peritoneal cavity of guinea-pig No. 1 at 1.52 P.M.

1.55. Muscular twitchings of head and neck.

2 P.M. Startings and twitchings continue.

It gives faint squeaks occasionally, as though the sudden startings which occur at intervals of 5 or 6 seconds cause pain.

2.5. Twitchings continue.

2.8. Very restless; twitchings going on, but no paralysis yet.

2.17. The same.

2.25. Restless and weaker; but still moves freely on being roused.

2.42. Sluggish; drags the hind legs.

2.58. Weaker; rolls partially over on one side, but can run when roused.

3.3. Lying on side, but can be roused; is partially paralyzed in hind legs. Respiration abdominal and hurried.

3.5. Nearly quite paralyzed; is roused with difficulty.

3.7. Can still be roused. Abdomen distended and painful; cries out when it is touched, as though peritonitis were setting in.

3.12. Can be roused with difficulty; respiration hurried; convulsive movements of fore legs and neck. Can still stagger for a few paces; but coordination of muscular power much diminished.

3.30. In violent convulsions.

3.38. Convulsions continue.

3.45. Quiet. Paralyzed; but reflex action still continues.

3.55. Dead in 2 hours and 3 minutes.

3.56. Electrodes in cord cause twitching of muscles of the back, and very slightly in those of the legs: the cord was evidently all but paralyzed. Muscular fibre contracts freely to direct stimulus of current. The intestines were ecchymosed and congested. There were effusions of red serum into the peritoneal cavity, and much ecchymosis of peritoneum and subperitoneal and intra-muscular areolar tissue. Peristaltic action continued faintly.

4 P.M. The heart has ceased to contract 4 minutes after apparent death ; it continued to contract, especially the auricles, for part (not the whole) of the time.

The blood removed from the heart-cavities and vena cava rapidly formed a firm coagulum in a glass receiver.

The electrodes applied to the sciatic showed that the nerve-trunk, as well as the spinal cord, was paralyzed.

#### *Experiment IV.*

Guineapig No. 2, an albino, had the  $\frac{1}{4}$ -grain cobra-virus solution injected into its peritoneal cavity at 1.56 P.M.

It immediately became much excited.

1.57. Is now quite tranquil.

2. Sluggish. Does not twitch as guineapig No. 1 did.

2.4. Started and squeaked slightly, as though in pain, but no twitching.

2.5. Slight twitching generally. Paralysis and ataxy commencing ; drags its legs with difficulty.

2.9. Sharp twitchings of head and neck.

2.12. Subsided on to the belly ; head fallen over ; crawls with difficulty ; is very feeble, almost paralyzed. The albino eyes have a heavy dull look ; lost their bright pink.

2.14. Convulsed.

2.15. Reflex action ceased. Apparently dead, but heart can still be felt beating. Occasional convulsive twitchings of lower lip.

2.16. Dead in 21 minutes.

2.17. All movements have ceased. Heart had ceased to contract, except slight flickering movements of auricles.

2.20. Electrodes in cord. Spinal cord and nerves paralyzed ; muscles contract freely to direct stimulus of current. Heart distended with blood. Blood, when removed, formed rapidly a firm coagulum. Intestines, peritoneum, and subperitoneal areolar tissue congested and ecchymosed. Sanguinolent effusions into peritoneum, but not so well marked as in the *Crotalus*-poisoning. Peristaltic action of bowels ceased rapidly.

The results of these two experiments show, so far, that the action of the cobra-poison is more energetic than that of the rattlesnake. Both were watery solutions of exactly the same quantity of the dried virus ; but it is to be borne in mind that that of the rattlesnake was  $6\frac{1}{2}$  years old, while that of the cobra was only one year old.

The guineapigs were both full-grown and of the same size ; yet one succumbed in 20 minutes to the cobra-poison, while the other survived the inoculation of the rattlesnake-poison for 2 hours and 3 minutes.

There were no very marked differences in the action of the poison in



these two cases, except in the energy with which the cobra exceeded the *Crotalus*.

*Crotalus.*

Twitchings; restless; squeaks; sluggish; ataxy; paralysis. Hurried respiration. Peritonitis. Convulsions. Death in 2 hours 3 minutes. Coagulated blood. Ecchymosis and extravasation of serous effusion well marked. Cord paralyzed. Muscles retain irritability.

*Cobra.*

Twitchings; excitement; squeaks; sluggish; ataxy; weakness; paralysis. Convulsions. Death in 20 minutes. Spinal cord and nerves paralyzed. Muscles irritable. Heart distended. Blood congested. Ecchymosis. Congestion less than in *Crotalus*.

*Experiment V.*

June 10th.—A grain of *Crotalus*-poison diluted with water was injected into the peritoneum of a full-grown guineapig at 2.40 P.M. Twitchings began almost immediately.

3.3. Restless; startings; staggers on hind legs.

3.20. Very weak, especially in hind quarters. General paralysis setting in. Abdomen distended and very tender.

3.30. In convulsions. Still feels when the abdomen is touched.

3.37. Paralyzed; but feels the touch. Reflex well marked.

3.45. Apparently dead in 65 minutes.

3.48. Cavities opened. Auricles flickering. Blood from heart and great vessels coagulated firmly. Abdominal cavity and areolar tissue and subperitoneal tissue infiltrated with bloody serum. Much ecchymosis of peritoneum and intestines, but not of lungs. Cord and nerves paralyzed. Muscles contract vigorously to induced current.

*Action of Crotalus-poison on Rabbit.**Experiment VI.*

$\frac{1}{4}$  of a grain (.015 gramme) of the same *Crotalus*-poison, dissolved in 1 cub. centim. of water.

The jugular vein of a large white rabbit was exposed, and the above solution was injected into it at 1.50 P.M.

At 1.51 violent convulsions, with opisthotonos.

At 1.53 apparently quite dead. Artificial respiration commenced immediately. Heart acting still, though feebly and with irregular flickering contractions. Spinal cord exposed. Electrodes applied; no reaction.

2.12. Heart still contracting feebly.

2.15. Faint contractions of heart still observable. Ventricles punctured, and blood withdrawn. Peristaltic action has ceased.

2.20. Feeble cardiac movements continue.

2.21. Heart has now ceased. Muscles react to direct current. Death

caused by rapid paralysis of medulla and cord. The blood taken from the heart and great vessels did not coagulate. At 4 P.M. it was still fluid, though very florid in colour.

Examined under the microscope nearly 2 hours after apparent death, the white corpuscles appeared natural; the red corpuscles not in rouleaux, and very much crenated, though a few retained their natural contour.

The blood was natural to test-paper.

### Experiment VII.

June 17th.— $\frac{1}{4}$  of a grain (.015 gramme) of dried cobra-poison, dissolved in 1 cub. centim. of water, was injected into the jugular vein of a large white rabbit, of the same size as in the previous experiment, at 2.55 P.M.

The rabbit passed at once into violent convulsions, and was apparently dead before it could be removed from the board, within one minute. The cord was immediately exposed, artificial respiration having also been begun. Electrodes applied, with strong current; no reaction; the cord was perfectly paralyzed.

Thorax examined at 2.59. Heart had ceased to contract. Ventricles moderately contracted. Auricles distended with blood. Phrenic irritated, quite paralyzed. Diaphragm, when directly irritated by current, contracts very faintly, whilst the neighbouring muscles contract vigorously. Peristaltic action goes on. Electrodes applied to vagus appear to accelerate peristaltic action; applied to splanchnic, they diminish it.

3.7. Ventricles of heart have now contracted firmly.

3.15. Blood taken from heart and great vessels has coagulated, but not firmly. The clot is small, and the serum very red.

3.15. Electrodes to sciatic; no reaction. Blood examined under microscope; no aggregation in rouleaux, no crenation of corpuscles. Blood neutral to test-paper.

We have in former papers remarked that when the cobra-poison was injected into the jugular vein directly and caused almost immediate death, that the fatal result was due to cessation of the heart's action by arrest in systole, and such was partially the case in the last experiment (VII.), made for the purpose of comparison with *Crotalus*; but in experiment VI. death was not so caused, for the heart continued to contract for about 28 minutes after apparent death, which was probably due to the sudden and total annihilation of the functions of the medulla and cord, no reaction to a strong current occurring when the electrodes were applied immediately after apparent death.

In this instance of *Crotalus*-poisoning it is also to be remarked that the coagulability of the blood was destroyed, whilst in that by cobra-virus it was only partially so.

It appears from the results of this experiment that the direct inoculation of large doses of the virus, whether viperine or colubrine, into the



circulation have the power in some cases of annihilating almost instantaneously the irritability of the cord and medulla, as in others they have of arresting the heart's action.

### Experiment VIII.

June 17th.—Ten drops of the blood of the rabbit described in the last experiment, poisoned by *Crotalus*-virus, were injected into a guineapig's thigh at 3.40 P.M.

The guineapig was not apparently affected constitutionally by the poisoned blood. It was alive the next morning; but the leg was swollen and discoloured. It ultimately recovered.

### Experiment IX.

June 24th, 1874.—A full-grown cat was chloralized at 1.20 P.M.  $\frac{1}{4}$  of a grain of *Crotalus*-poison, diluted with 1 cub. centim. of water, was injected into the jugular vein. The respirations were immediately quickened.

1.21. Twitching of muscles generally.

1.22. Efforts to vomit. Forcible extension of limbs.

1.24. Hurried respiration and retching. Reflex action perfect.

1.30. Muscular twitching and tetanic stretching of limbs. Efforts to vomit continue. Micturition. Rolls over on the ground.

1.34. Ataxy. Staggers when walking, which it can only do for a few paces. Peculiar twitching of diaphragm; not synchronous with respiratory movements. Rolls over on its side.

2 P.M. In the same state.

2.8. Injected  $\frac{1}{8}$  of a grain more of the poison into the same jugular vein. The animal immediately got up and walked, comparatively steadily, for several paces, as though it had been stimulated, and then rolled over.

2.16. Twitching of diaphragm continues at the rate of 150 per minute.

2.18. Again got up and walked for a few paces; but it is gradually becoming more paralyzed.

2.44. Violent tetanic spasms of limbs. Reflex action diminished.

2.46. Reflex action gone from eyes. Deep sighing respiration.

2.47. Convulsions. Death. Body opened immediately. Lungs deeply congested and much ecchymosed. Deep red gelatinous effusion all about the roots of the lungs. Heart contracting. Electrodes applied to phrenic caused vigorous contraction of diaphragm.

2.50. Heart ceased to contract 3 minutes after respiration had ceased.

2.52. Electrodes in cord; do not cause contraction of limbs.

2.54. The sciatic nerve, when irritated, conveys impressions; muscles of legs contract. Blood from the heart and great vessels did not form a coagulum, and remained permanently fluid. Red corpuscles of blood were much crenated.

Death in this case appeared to be caused through the medulla.

*Experiment X.*

*June 15th*, 1874.—Action of *Crotalus*-poison on the frog.

A frog's hind leg was ligatured excluding the sciatic nerve.

A solution of *Crotalus*-poison was injected into the lymph-sac at 12.32

P.M.

2.30. Sluggish, but not otherwise affected.

3.15. In the same condition.

*June 16th*.—12.3, noon. Sluggish, but can still move.

*June 17th*.—Found dead this morning early; pupils contracted.

Electrodes applied; no reaction in either cord or nerves on either side to the strongest current.

The frog may have been dead some hours.

*Experiment XI.*

*June 15th*.—At 3 P.M. same day a solution of *Crotalus*-venom was injected into the dorsal lymph-sac of a frog, the aorta having been previously ligatured, so as to prevent the poison from affecting the trunks or peripheral extremities of the sciatic nerves.

3.40. The frog seems quite unaffected.

*June 16th*.—12.30, noon. Frog dead; not rigid; mouth open.

Irritation of cord with strongest current does not cause contraction of legs. Irritation of sciatic with coil at 24 causes twitchings of gastrocnemius.

Neither of these two experiments give any definite results, as the period intervening between death and examination of the condition of the nerve-centres was not determined exactly.

The results of the following experiments show that the local as well as the general effect of the cobra- and *Crotalus*-poisons, *i. e.* colubrine and viperine, is to cause hæmorrhage, ecchymosis, and sanguinolent effusions into the areolar tissue, not only at the seat of inoculation and its neighbourhood, but also in the mucous membranes and other vascular parts. It is obvious also that the *Crotalus*-poison acts more energetically in this respect than the cobra-poison, and that this is perhaps one of the most marked distinctions between them.

*Experiment XII.*

*August 6th*, 1874.—A cat was chloralized, and part of the mesentery placed under the microscope on the warm stage. *Crotalus*-poison, diluted with water, was then applied to the mesentery, and its effects watched. The white corpuscles were observed to cling in quantities to the walls of the vessels, and as the current of blood hurried through them, some masses of pale matter, like aggregation of white corpuscles, were observed to pass with the stream; very soon, marked extravasation of red corpuscles took

place, and to the naked eye the mesentery became discoloured by patches of ecchymosis in the course of the small blood-vessels, like the foliage on the branches of a tree.

There could be no doubt that the local action of the poison had a marked effect in producing extravasation of blood.

### *Experiment XIII.*

A similar experiment was repeated on another part of the mesentery of the same cat with cobra-poison, exactly as the *Crotalus*-poison had been applied in the previous experiment. This was carefully watched, but no extravasation took place; there was a marked difference in the result of the application of the two poisons, at all events as far as these two experiments were concerned.

### *Experiment XIV.*

August 12th, 1874.—A cat was chloralized at 2.30 P.M. Mesentery exposed and placed under microscope on warm stage.

*Crotalus*-poison applied to mesentery; circulation soon diminished in some vessels but continued vigorously in others. Isolated extravasated patches soon made their appearance of a triangular form, others followed and coalesced with these until a network was formed in the course of the vessels all over the field. The extravasation soon became general, the circulation still continuing slowly.

### *Experiment XV.*

A fresh portion of mesentery of same cat exposed. Intestines becoming cold and circulation now very languid.

Cobra-poison applied.

No apparent effect produced; but the circulation is very languid, indeed has almost ceased, so that the results of this experiment are not conclusive.

### *Experiment XIV.*

August 14th, 1874.—A cat was chloralized, part of mesentery withdrawn, and placed under microscope on warm stage.

Dried cobra-poison dissolved in a salt solution, .75 per cent., applied to the mesentery at 4.10 P.M.

4.14. Circulation is languid, almost ceased in some vessels.

4.18. Slight extravasation taking place where the poison has been in contact.

4.20. Extravasation rather more obvious.

4.35. Exposed another part of the mesentery; examined the state of the circulation before applying the poison. Blood flowing languidly.

Poison applied at 4.37; at first it seemed rather to accelerate the movement of the blood.



4.38. Circulation continues at same rate.

4.42. Same rate.

4.45. It becomes more languid.

4.48. Circulation has ceased, but yet there is no marked extravasation.

#### *Experiment XVII.*

Another portion of the same mesentery had cobra-poison applied, but after half an hour there was no sign of extravasation.

#### *Experiment XVIII.*

A fresh piece of mesentery exposed of same cat, and diluted *Crotalus*-poison applied at 4.52 P.M.

The circulation was rather languid at the time, and apparently became more languid.

At 4.58 no extravasation had taken place, the blood flowing very languidly.

5.15. Circulation still going on, but very slowly; no extravasation; it soon after ceased.

#### *Experiment XIX.*

At 5.20 P.M. a fresh portion of the mesentery was exposed; to one part cobra- and to the other *Crotalus*-poison was applied, and the effect was watched with the naked eye.

5.45. No extravasation visible.

At 6.15 P.M. slight extravasation equally visible on both.

#### *Experiment XX.*

August 25th, 1874.—At 2 P.M. a young cat was chloralized. The mesentery was drawn out and a part treated with cobra-poison, another part with *Crotalus*-poison.

At 5 P.M. On examination, that under the influence of the *Crotalus*-poison was found deeply congested and reddened with blood, extravasated in the course of the small vessels, forming a well-marked redness to the naked eye. Under the microscope the red corpuscles were seen in numbers outside the vessels. Circulation still going on vigorously. That part treated with cobra-poison was barely altered, but, on close examination, slight patches of extravasation were seen in the course of the vessels.

The difference was well marked between the two—the extravasation produced by *Crotalus*-venom being well marked, that by cobra-venom scarcely perceptible. In both cases the microscope showed red corpuscles outside the vessels.

These experiments show that *Crotalus*-poison causes hæmorrhage and hæmorrhagic effusions more than the cobra-poison does.

The following experiments were made, at the suggestion of Mr. Darwin,

with the object of testing the influence of snake-poison on ciliary action, especially in reference to its comparative action on vegetable protoplasm, as will be seen by his remarks.

### *Experiment XXI.*

#### *Influence of Cobra-poison on Ciliary Action.*

*June 29th, 1874.*—Ciliated epithelium from the frog's mouth was treated with a solution of cobra-poison and examined under the microscope.

At 1.35 P.M., when examined, the action of the cilia was vigorous.

At 1.45 it was much diminished.

At 1.55 it had entirely ceased.

### *Experiment XXII.*

Ciliated epithelium placed under microscope; one part was treated with water, the other with the poisoned solution.

At 2.10 P.M. ciliary motion vigorous in both, perhaps more so in that subjected to the poisoned solution.

2.18. Non-poisoned cilia active. Poisoned cilia very feeble.

2.20. Non-poisoned cilia still active. Poisoned cilia very feeble.

2.24. Non-poisoned cilia active. Poisoned cilia very languid.

2.30. Non-poisoned cilia still active. Poisoned cilia have entirely ceased to act.

It is evident from this that the poison first stimulates and then destroys the activity of the ciliary action.

### *Experiment XXIII.*

*August 14th.*—Frog's blood placed in salt solution, .75 per cent., at 1.25 P.M. on warm stage, and then subjected to the action of cobra-poison.

At first the amœboid movements of white corpuscles went on vigorously. At 2 P.M. they had ceased, or very nearly so, in all that appeared in the field.

2.30. All movement had entirely ceased. The red corpuscles seemed more flattened, the nucleus more visible, and the edges better defined, assuming a pointed and more oval form than usual.

### *Experiment XXIV.*

*August 25th, 1874.*—Newts' blood examined under  $\frac{1}{8}$  object-glass on hot stage, white corpuscles moving slowly. Cobra-poison applied, but no perceptible change observed.

The following communications were received from Mr. C. Darwin on the action of some of the same cobra-poison on vegetable protoplasm:—

“You will perhaps like to hear how it acted on *Drosera*. I made a solution of  $\frac{1}{4}$  gr. to 3ij of water. A minute drop on a small pin's head

acted powerfully on several glands, more powerfully than the fresh poison from an adder's fang.

"I also immersed three leaves in 90 minims of the solution; the tentacles soon became inflated and the glands quite white, as if they had been placed in boiling water. I felt sure that the leaves were killed; but after 8 hours' immersion they were placed in water, and after about 48 hours reexpanded, showing that they were by no means killed. The most surprising circumstance is, that, after an immersion of 48 hours, the protoplasm in the cells was in unusually active movement. Now, can you inform me whether this poison, if diluted, arrests the movement of vibratile cilia?"

"I dissolved  $\frac{1}{2}$  gr. [of cobra-poison] in 3j of water, so that I was able to immerse two leaves. It acted as before, but more energetically; and I observed more clearly, this time, that the solution makes the secretion round the glands cloudy, which I have never before observed. But here comes the remarkable point; after an immersion of 48 hours, the protoplasm within the cells incessantly changes form, and I never saw it on any other occasion so active. Hence I cannot doubt that this poison is a stimulant to the protoplasm; and I shall be very curious to find out in your papers whether you have tried its action on the cilia and on the colourless corpuscles of the blood. If the poison does arrest their movement, it will show that there is a profound difference between the protoplasm of animals and of this plant. Therefore if you try any further experiments I hope that you will be so kind as to inform me of the results. I may add that I tried at first 1 gr. to the 3j, as that is my standard strength for all substances.

"It is certainly very remarkable that the poison should act so differently on the cilia and on the protoplasm of *Drosera*. After the 48 hours' immersion, I placed the two leaves in water and they partially reexpanded. I thought that the whitened glands were perhaps killed; but those of one leaf which I tried with carbonate of ammonia absorbed it, and the protoplasm was affected in the usual manner. I am very much surprised at the action of the poison on the viscid secretion from the glands, which it coagulates into threads and bits of membrane, with much granular matter. Have you observed whether the poison affects in any marked manner mucus or other such secretions?"

### *Experiment XXV.*

#### *Action of Cobra-poison on Muscle.*

June 29th, 1874.—A standard solution of cobra-poison, .03 gramme to 4.6 cubic centims. of water, was prepared.

1.25 P.M. The gastrocnemius of a frog was separated and immersed in this solution in a watch-glass; it immediately contracted considerably.

1.30. The muscle contracts with current at 11.



1.45. The muscle has lost its irritability; does not respond to the strongest current.

*Experiment XXVI.*

At the same time (1.25 P.M.) the gastrocnemius from the other leg of the same frog immersed in water. Did not immediately contract like that placed in the poisoned solution.

1.30. Contracts strongly to current at 15 c. m. of Du Bois Reymond's coil, more than the poisoned muscle at 11, at the same moment.

1.45. Contracts distinctly at 11, whilst the poisoned muscle has lost all irritability.

From this it is evident that the poison first stimulates the muscular fibre to contract, but rapidly afterwards destroys its irritability.

*Experiment XXVII.*

The gastrocnemii of a frog were again treated in the same way as in the previous experiment, with precisely the same results.

June 28th.—Made several experiments with cobra-poison on ciliated epithelium of frog's mouth, and found that it at first accelerated, then destroyed, the action of the cilia.

*Experiment XXVIII.*

*To test the effects of Cobra-poison, when swallowed, on the Frog.*

June 24th, 1874.—At 2.25 P.M. about  $\frac{1}{8}$  of a gr. of dried cobra-poison was passed down a frog's throat.

2.30. Frog making violent efforts to vomit. Gaping. Head thrown back tetanically.

2.34. Bloody mucus vomited with violent efforts \*.

2.50. Moves with difficulty; is becoming paralyzed. Efforts to vomit continue.

3. Much the same.

3.5. Very weak; still tries to vomit.

3.10. Reflex action still well-marked.

3.15. Motor nerves apparently quite paralyzed.

3.20. Apparent death.

*Artificial Respiration with pure Oxygen.*

As life had been prolonged for many hours in snake-poisoning by artificial respiration with atmospheric air, it was thought expedient to ascertain if the more complete oxygenation by the undiluted gas would be more efficacious, as it seemed might be possible; accordingly the following experiment was made on the 24th April, 1874.

\* This experiment is especially interesting, as showing that frogs do occasionally vomit, a fact which has been denied by some physiologists.

*Experiment XXIX.*

$\frac{1}{4}$  of a grain of dried cobra-poison dissolved in distilled water was injected into a rabbit with the hypodermic syringe.

Symptoms of poisoning were rapidly manifested. A tube had been previously introduced into the trachea, and respiration was commenced as soon as poisoning was manifest.

Artificial respiration, with oxygen contained in a large bag, was steadily continued for two hours, but with no better effect than in other similar cases where atmospheric air was used for the same purpose. At the expiration of two hours, apparent death had occurred; the heart continued to beat for about two minutes after the respiration ceased.

Beyond a very florid condition of the blood, there was no obvious difference between the effect of oxygen and that of common air. It did not indeed appear that, as far as the effects produced by the poison were concerned, it differed in its action from common air.

*Experiment XXX.*

November 1874.—A little cobra-poison, dissolved in water, was added to water containing some cells scraped from the mantle of a freshwater mussel. Among these was a large ciliated cell, which, before the addition of the poison, had been moving slowly, although its cilia were moving actively. Immediately after the addition of the poison the cell began to spin round on its own axis with extraordinary rapidity. In about three or four minutes its motions began to be languid, the ciliary motion ceased, the cell itself elongated, contracted, and then slowly resumed its former shape and became perfectly motionless.

*Experiment XXXI.*

Water from the interior of a freshwater mussel, and containing two specimens of *Paramœcium* in active motion, was examined. They were rotating with great rapidity. A little cobra-poison diluted with water was added. Three minutes after the addition one was discovered with both the cilia and cell-body perfectly still. The cilia of the other were still, but the cell-body was contracted. In about half a minute more it expanded to its normal size and then remained perfectly still.

*Experiment XXXII.*

A piece taken from the mantle of a freshwater mussel was placed on the slide and examined at the end of about half an hour. Active ciliary motion could be observed both in the fringe of the mantle itself and in several specimens of *Paramœcium*. A little dilute poison was added. At first the ciliary motion seemed increased, but in about two minutes it became slower, and in six had become very languid, and in ten minutes stopped altogether in the specimens of *Paramœcium*, but still continued in some of the cilia of the mantle.

*Experiment XXXIII.*

A little dilute cobra-poison was added to a piece of the mantle of a freshwater mussel. The cilia began immediately to move much more rapidly. This was watched for some time. Ciliary motion not affected, or at all events not arrested, after more than half an hour.

*Experiment XXXIV.*

December 10th, 1874.—A piece of the gills of a freshwater mussel placed under the microscope and a little cobra-poison added at 10.40 p.m. The cilia were extremely active.

At 10.55 still active.

11.5. Several ciliated amœboid masses are now quiet instead of rolling over and over as they did, but the cilia on their surface are still moving.

11.15. The cilia on these Infusoria have now nearly all stopped. A few are moving slowly, whilst those on the gills are but little affected.

11.55. Cilia on the gills are still quite active. Those on the ciliated bodies still moving, rather more actively than before.

1.30. Cilia on gills have become much more sharply outlined. Many are standing still, though many still move briskly.

*Experiment XXXV.*

To another specimen a strong solution of cobra-poison was added at 10.50.

1.30. Cilia still moving.

*Experiment XXXVI.*

A third specimen was laid in an almost syrupy solution of dried cobra-poison at 11.28.

At 11.40 no effect observable.

1.30. Some have stopped, but numbers are still moving quite briskly.

In this case the poison seemed not to have any action on the ciliary motion.

*Experiment XXXVII.*

January 6th, 1875.—At 3.40 some diluted cobra-poison added to *Vallisneria*. Circulation going on vigorously. About  $\frac{1}{10}$  grain in three drops of water.

3.58. The movements are unchanged.

5 p.m. Movements going on as before.

*Experiment XXXVIII.*

Added some solution of cobra-poison at 4 p.m. to another specimen of *Vallisneria*.

4.10. No change.

4.45. Circulation goes on vigorously.

4.55. Perhaps rather less brisk in their movements.



The results of these experiments show that cobra-virus must be regarded as, to a certain extent, a poison to protoplasm, seeing that it arrested with rapidity the movements in Infusoria\* (*vide* Experiments XXX., XXXI., and following). Still it cannot be regarded certainly as a very powerful one, for the cilia of the freshwater mussel continued to move for many hours in a strong solution of cobra-poison; though in other experiments the action was apparently arrested even in weaker solutions of the poison. In the case of cilia from the frog's mouth, the results were more definite, but action was not invariably destroyed. The results of the action of the poison on the amœboid movements of the blood-corpuscles are not very definite. In the case of *Vallisneria*, the circulation in the cells went on with undiminished vigour after the application of the poison for two hours.

\* Is this accounted for by the existence of a rudimentary nervous system diffused throughout these two forms of life, and on which the poison could act?